***Objection to the claims***

Applicants herein correct the minor misspelling of "cytoskeletal." Thus, the Examiner's objection is overcome.

***Rejection under 35 U.S.C.§101***

The Examiner rejects claims 25-27 as directed to unpatentable subject matter because they include humans. Applicants herein change the claim language to recite "mammalian" or "mammal" as appropriate and to include the recitation "non-human" thereby excluding the unpatentable subject matter and obviating this rejection.

***Rejection under 35 U.S.C. §112, first paragraph***

The Examiner rejects claims 1-24 and 28-38 because the claim language encompasses cross-species transfer. Applicants herein change the claim language to recite "mammalian" cells and animals produced therefrom as are the cells specifically exemplified in the specification. The Examiner has already admitted that cloning was predicatable in sheep, cows and rodents as discussed further, *infra*. Therefore, Applicants remove species outside the category of mammals. Even if, *assuming arguendo*, cloning was not predictable in all species of mammals, the Examiner is reminded that the patent law is clear that a genus is not legally defective merely because it includes inoperable embodiments.

The Examiner further challenges enablement on other bases alleging generally that "techniques of nuclear transfer" were unpredictable at the time of filing. The Examiner cites to the following references for support for this proposition:

1. Westhusin
2. Polejaeva
3. Pennisi
4. Mitalipov

5. Simerly
6. Meirelles

Applicants believe that the Examiner's comments make the following basic allegations:

1. Cloning even within "mammals" was allegedly unpredictable at the time of filing the patent application;
2. There is variability between the species on what is required for successful cloning therefore requiring extensive experimentation (alleging only the cloning of sheep, cows and rodents was predictable);
3. A majority of embryos implanted into the womb allegedly result in miscarriages;
4. Embryonic cells have been successful in some instances, but there is allegedly no report of a successful cloning using somatic cells;
5. Nuclear transfer across species is allegedly probably not possible as the nuclear material may not be compatible with the host cell (*citing* Meirelles), and even if it is, there may be incompatibility between the embryo and host animal (*citing* Fehilly *et al.*).

In order to advance prosecution, Applicants herein add the recitation "mammalian" into the claim language thereby removing additional species outside the category of mammals from the scope of the claims. Even if, *assuming arguendo*, cloning was not predictable in all species of mammals, the Examiner is reminded that the patent law is clear that a genus may include inoperable embodiments and not every species within the scope of a legally acceptable genus must be specifically enabled.

In addition, Applicants respectfully submit that, *assuming arguendo,* nuclear transfer across species is impossible, a statement for which the Examiner supplies no evidence, the same principles of the patent law apply. That is, the patent law as regards enablement is clear that inoperable species and species that are not specifically enabled may exist within the scope of a genus that is legally defined within the bounds of 35 U.S.C. 112, first paragraph.

As regards the remaining points, namely 1, 3 and 4, Applicants respectfully submit that animals have been successfully cloned using the presently claimed methods. The Examiner's attention is directed to the enclosed abstract of Applicant's own work. The literature clearly supports the patentability of the present invention as the enclosed abstract demonstrates. Applicants submit their willingness to submit a copy of the complete manuscript detailing such successful cloning as soon as it is completed.

Applicants respectfully remind the Examiner that the law does not require absolute certainty, predictability or certainty to fulfill the requirements of the patent statute as regards enablement. In fact, the courts have specifically stated that a great deal of experimentation is acceptable before the threshold to undue experimentation is passed thereby leading to a finding a non-enablement. *See, e.g. In re Wands, 858 F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1998).* As is the case with the instant invention, one of ordinary skill in the art could use the teachings of this specification to reproduce successfully cloned organisms without undue experimentation. It would simply be a matter of routine screening at every step to determine whether the nuclear transfer was successful and whether the embryo, fetus and animal was growing and developing. Such routine screening has been found to provide an enabling disclosure.

***Rejection under 35 U.S.C. §112, second paragraph***

The Examiner indicates that claim 19 does not further limit claim 10 and should be cancelled. Applicants herein cancel claim 19 as suggested in the interest of advancing prosecution and securing rapid allowance of a patent.

***Rejection under 35 U.S.C. §102(b)***

The Examiner rejects all of claims 1-38 under 35 U.S.C. §102(b) on the basis of one or more of the following three references.

1. Bordignon *et al.*, *Molec. Reprod. Devel.* 49, 29-36

The Examiner rejects claims 1-18 as anticipated by Bordignon *et al.* According to the Examiner, Bordignon *et al.* teach methods of preparing a reconstituted nonhuman oocyte and methods of reconstituting a nonhuman embryo by activating an MII oocyte by ethanol treatment, permitting the oocyte to mature to telophase II where there is an extrusion of the second polar body, enucleating the telophase II oocyte in the presence of cytochalasin B by removing the nucleus and a portion of the surrounding cytoplasm using a micropipette, electrofusing a blastomore isolated from an IVF 5.5 day blastocyst to introduce a germinal cell nucleus into the enucleated oocyte and culturing *in vitro* the reconstructed embryo into a blastocyst. Allegedly, the blastomoeres are inherently in G1, S, G2 or M stage since the blastomeres are actively dividing. Moreover, IVF inherently causes the blastomere to be cultured.

Applicants respectfully submit that Bordignon *et al.* teach a telophase enucleation technique to produce cloned animals from early embryonic blastomeres. Bordignon *et al.* do not teach or suggest cloning of animals using cells other than early embryonic blastomeres. As is the case with the presently claimed invention, a period of culture is performed when using donor nuclei from cells ***other than*** embryonic blastomeres. The period of culture before transfer into enucleated

oocytes is performed to starve the cells as shown on page 12, lines 3 to 5 of the instant specification, or for reprogramming the cells in the cytoplasm of an incactivated oocyte as disclosed on page 1, lines 33 to 35. It was of general belief in the prior art (as mentioned on page 5, lines 1 to 5 of the instant specification) that an oocyte could not be activated before enucleation and receive nuclei from cultured cells of embryonic, germinal and somatic origin. The presently claimed methods therefore are a clear advancement over the teachings and beliefs of the prior art. There was simply no teaching or suggestion in the prior art that an oocyte could successfully be activated before enucleation and receive nuclei from cultured cells of embryonic, germinal and somatic origin.

As further evidence of the novelty and patentability of the present invention, Applicants respectfully direct the Examiner's attention to page 35, last paragraph of Bordignon *et al.* wherein it is stated "it is not possible to determine which elements of the protocol resulted in beneficial developmental effects with the proposed protocol." This statement clarifies the inventive nature of the instant invention as it clarifies that it was not anticipated that the methods currently claimed could be performed.

2. Cibelli *et al.*, *Theriogenology* 49:236

The Examiner rejects claims 25-27 as anticipated by Cibelli *et al.* According to the Examiner, Cibelli *et al.* teach transgenic mammalian embryos, fetuses and adults. The Examiner invites Applicants to prove that the products produced in the claimed process are different from the transgenic embryos, fetuses and adults from the cloning work of Cibelli *et al.* In accordance with United States patent law, "product by process" claims are not patentable unless there is some novel feature of the product obtained by a patentable process.

Applicants respectfully submit that one of skill in the art will readily recognize that the product of claims 25-27 is indeed quite different from the product of Cibelli *et al.* Cibelli *et al.* perform cloning of fibroblast nuclei by introducing such into an

enucleated oocyte. The resulting reconstituted embryos are then grown to the blastocyst stage. Blastomeres from these blastocysts are then plated to generate embryonic stem cell lines. In order to test the pluripotency of these cells *in vivo*, embryonic stem cells are injected into 8 to 16 cell mammalian embryos which are themselves transferred into recipient cows. The offspring or calves resulting from these transfers are characterized by being chimeric, having dispersed cloned embryonic stem cells in their body. Therefore, the organisms of Cibelli *et al.*, are composed of a mixture of cells mainly originating from an embryo with some cloned stem cells transferred therein prior to implantation in a surrogate mother.

In the case of the present invention, the embryos, fetuses and offspring are obtained from a unique transgenic cell. Therefore, all cells of the embryos, fetus and offspring of claims 25-27 are composed ***entirely*** of cells originating from only one cell. That is simply not the case of the product obtained by the methods of Cibelli *et al.*

3. Echelhard, U.S. Patent 6,580,017 B1

The Examiner rejects claims 1-38 as anticipated by Echelhard. The Examiner contends that Echelhard teaches methods for producing reconstructed goat oocytes, reconstituted goat embryos, methods for producing transgenic goat embryos, and methods of cloning a goat comprising activating an oocyte by incubation in 7% ethanol (*citing* Col. 19, lines 15-19), incubating the activated oocyte to telophase II and then further incubating the oocyte in the presence of cytochalasin B, enucleating the activated, telophase II oocyte by aspiration, transferring a cultured goat fetal fibroblast which contains a DNA sequence encoding antithrombin III into the perivitiline space of the enucleated oocyte, fusing the reconstructed oocyte by electrofusion, and culturing the reconstituted oocyte to produce a transgenic embryo

which is then transferred to a surrogate mother. According to the Examiner, the fibroblast donor cells were inherently in one of G0, G1, S, G2 or M stages.

Applicants respectfully submit that Echelhard teaches using ethanol for treating oocytes. This provides negative results when cloning the fibroblast cells. The results presented in Table 2 of Echelhard demonstrate that oocytes treated with ethanol in telophase failed to give valuable embryos and fetuses after transfer. No embryos, fetus or offspring were found still living or in development. In addition, Applicants respectfully direct the Examiner's attention to Column 22, lines 34 and 35 of Echelhard wherein it is taught that no pregnancies were observed with embryos generated by the ethanol enucleation/activation protocol. Hence, in short the procedure of Echelhard does not work and is not operable. It therefore cannot anticipate the presently claimed invention.

Even further, Echelhard does not teach or suggest enucleation of activated oocytes performed precisely when undergoing expulsion of a second polar body or after the activated oocyte has expelled the second polar body. Such is the case with the methods of the present invention.

***Rejection under 35 U.S.C. §103***

Bordignon *et al.*, Molec. Reprod. Devel. 49, 29-36 in view of Cibelli *et al.*, *Theriogenology* 49:236

The Examiner rejects claims 1 and 19-38 over Bordignon *et al.* in view of Cibelli *et al.* The Examiner maintains that Bordignon suggests or motivates one of skill in the art to try using enucleated telophase oocytes by stating that when compared to the use of metaphase oocytes, telophase oocytes permitted the formation of blastocyst-stage embryos that had a larger number of cells (*citing*, page 35, column 1, paragraph 1, lines 1-5). Cibelli *et al.* teach producing transgenic bovines using somatic cells. The Examiner contends that motivation for producing

transgenic bovines by nuclear transfer comes from a need in the art to produce genetically identical individuals for husbandry purposes.

Applicants respectfully reiterate that Bordignon et al. teach a telophase enucleation technique to produce cloned animals from early embryonic blastomeres. Bordignon et al. do not teach or suggest cloning of animals using cells other than early embryonic blastomeres. Moreover, it was of general belief in the art (as mentioned on page 5, lines 1 to 5 of the instant specification) that an oocyte could not be activated before enucleation and receive nuclei from cultured cells of embryonic, germinal and somatic origin as explained, *supra*. Cibelli *et al.* perform cloning of fibroblast nuclei by introducing such into an enucleated oocyte.

In view of the foregoing, the Examiner has not set forth a proper *prima facie* case of obviousness. First, the Examiner will appreciate that even if the two references are combined, the instant invention is not attained. Neither reference teaches or suggests that a host oocyte may be activated prior to enucleation. Likewise, neither reference teaches enucleating the activated oocyte when the activated oocyte is undergoing expulsion of a second polar body or has expelled a second polar body. Second, the Examiner alleges a motivation to try using telophase enucleated oocytes, but a motivation to try to does not constitute a proper *prima facie* case of obviousness. The patent law is clear that a *prima facie* case of obviousness must include a motivation to try and a reasonable expectation of success. There can be no reasonable expectation of success in view of the teachings of the prior art that an oocyte could not be activated before enucleation and receive nuclei from cultured cells of embryonic, germinal and somatic origin (*See*, page 5, lines 1 to 5 of the instant specification).

**Fees**

No additional fees are believed to be necessitated by the instant Response. However, should this understanding be erroneous, authorization is hereby given to charge Deposit Account No. 11-1153 for any underpayment, or to credit any overpayments.

## III. CONCLUSION

Applicants respectfully request entry of the foregoing Amendments and Remarks into the file history of the instant Application. The Claims as amended are believed to be in condition for allowance, and withdrawal of all of the outstanding rejections is therefore believed in order. Early and favorable action on the claims is earnestly solicited. Should a discussion be helpful in resolving any outstanding issues, the Examiner is invited to telephone the undersigned at (201) 487-5800.

Respectfully submitted,

KLAUBER & JACKSON

J. David Smith
Attorney for Applicants
Registration No. 39,839

KLAUBER & JACKSON
411 Hackensack Avenue
Hackensack NJ 07601
Tel: (201) 487-5800

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# BOVINE EMBRYOS RECONSTRUCTED WITH SOMATIC CELLS ARRESTEDAT G0/G1 WITH METAPHASE OOCYTES OR CYCLING WITH TELOPHASE HOST OOCYTES SHOW SIMILAR DEVELOPMENTAL POTENTIAL

V. Bordignon and L.C. Smith
CRRA, Faculte de medecine veterinaire, Universite de Montreal, Saint-Hyacinthe, QC, Canada J2S 7C6

The cell cycle stage of donor nuclei and host cytoplasts are known to affect nucleo:cytoplasmic interaction and chromatin remodeling in embryos reconstructed by nuclear transplantation. Whether chromatin is believed to be better remodeled after exposure to metaphase-arrested (M) cytoplasts G0/G1-arrested (A) donor nuclei enable compatible nucleo:cytoplasmic interactions and, therefore, the A/M combination seems to be preferred in most cloning experiments using somatic cells. We hypothesize that cytoplasts obtained from telophase-enucleated (T) oocytes are able to remodel chromatin from cells that are cycling (C) at the time of reconstruction and, thereby, the C/T combination eliminates the harmful effects of incompatible nucleo:cytoplasmic interactions with proper chromatin remodeling. Therefore, the main objective of this study was to compare the developmental potential of A/M vs. C/T-reconstructed embryos. Fetal fibroblast (FF) and adult granulosa (GC) primary cell cultures were established in medium alpha-DMEM supplemented with antibiotic and 10 % FCS. Preliminary assays were conducted to determine the cell cycle phases by recovering and analyzing cells at different periods after plating using flow cytometry. The percentage of cells at the G0/G1-phase was 90 and 91, 97 and 95, 41 and 31, 47 and 66, 46 and 64, 83 and 77, 96 and 93, 98 and 96, 97 and 95, 97 and 93 for FF and GC at 0, 8, 16, 24, 32, 48, 72, 96, 120 and 144 h post plating. Based on these results, cells for the A/M combination were used after several days of confluence and after 16 to 24 h from plating for the C/T group. For the A/M group, oocytes were enucleated at 22 h, fused at 26 h and activated at 28 h from the beginning of in vitro maturation (IVM). For the C/T group, oocytes were activated at 28 h after IVM and enucleated and fused 2.5 h later. Electrofusion was performed by applying a single 1.7 KV/cm 70 μsec electric pulse using a 0.3 M manitol solution. Oocyte activation was induced by exposure to 5 μM Ionomycin in TCM-199 hepes-buffered medium during 4 minutes. Reconstructed embryos were cultured for 8 days in SOF medium at 38.5 °C in an atmosphere of 5% $CO_2$ and 5% $O_2$ and transferred to synchronized recipient heifers whenever required. Development to the blastocyst stage for GC and FF reconstructed embryos was 19.3% (n=378) and 34.8% (n=108) for A/M and 24.1% (n=169) and 29.7% (n=43) for C/T (P>0.05). Preliminary studies with the A/T combination produced 3.1% (n=73) blastocysts development and 11.9% (n=50) development with a C/M combination. After transfer of two embryos per recipient heifer, pregnancy obtained at day 30, 60 and 120 for GC cells were 4, 2 and 0 (n=13) for A/M, 5, 3 and 0 (n=10) for C/T, and for FF cells 3, 2 and 1 (n=5) for A/M and 3, 3, and 3 (n=5) for C/T. These results indicate that cycling nuclei transferred to preactivated telophase-II enucleated oocytes show similar developmental potential to the combination of G1/G0-arrested nuclei with metaphase enucleated oocytes, suggesting that complete chromatin remodeling of somatic cells can be achieved in different cell cycle environments.

M. MARC

FAX: 418 640 1500